ISSN 0026-2617, Microbiology, 2006, Vol. 75, No. 3, pp. 329–335. © Pleiades Publishing, Inc., 2006.
Original Russian Text © I.S. Kulichevskaya, T.A. Pankratov, S.N. Dedysh, 2006, published in Mikrobiologiya, 2006, Vol. 75, No. 3, pp. 389–396.

EXPERIMENTAL ARTICLES

Detection of Representatives of the *Planctomycetes* in *Sphagnum* Peat Bogs by Molecular and Cultivation Approaches

I. S. Kulichevskaya, T. A. Pankratov, and S. N. Dedysh[1]

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received October 11, 2005; in final form, December 13, 2005

Abstract—By means of fluorescence in situ hybridization with 16S rRNA-targeted oligonucleotide probes (FISH), it has been shown that members of the phylum *Planctomycetes* represent a numerically significant bacterial group in boreal *Sphagnum* peat bogs. The population size of planctomycetes in oxic layers of the peat bog profile was in the range of $0.4–2.0 \times 10^7$ cells per g of wet peat, comprising 4 to 13% of the total bacterial cell number. A novel effective approach that combined a traditional cultivation technique with FISH-mediated monitoring of the target organism during the isolation procedure has been developed for the isolation of planctomycetes. Using this approach, we succeeded in isolating several peat-inhabiting planctomycetes in a pure culture. Sequencing of the 16S rRNA genes from two of these isolates, strains A10 and MPL7, showed that they belonged to the planctomycete lineages defined by the genera *Gemmata* and *Planctomyces*, respectively. The 16S rRNA gene sequence similarity between strains A10 and MPL7 and the phylogenetically closest organisms, namely, *Gemmata obscuriglobus* and *Planctomyces limnophilus*, was only 90%. These results suggest that the indigenous planctomycetes inhabiting *Sphagnum* peat bogs are so far unknown organisms.

DOI: 10.1134/S0026261706030155

Key words: the phylum Planctomycetes, *Sphagnum* peat bogs, fluorescence in situ hybridization, *Gemmata, Planctomyces.*

The *Planctomycetes* is a distinct phylum within the domain *Bacteria.* It is considered one of the most unusual and mysterious groups of prokaryotic microorganisms [1, 2]. Members of this group are budding organisms that possess a number of features uncommon to other bacteria. One of these major distinctive features is the absence of peptidoglycan in the cell-wall composition [3], which makes planctomycetes similar to chlamydiae and mycoplasmas. Another unique feature of a planctomycete cell organization is the presence of a membrane-defined compartment that contains a fibrillar nucleoid [4]. Moreover, in representatives of the genus *Gemmata*, a nuclear body is surrounded by a double membrane [5], a feature characteristic of eukaryotic cells only. Planctomycetes possess the largest genomes known in the *Bacteria* (up to 9 Mb). Genome analysis of *Rhodopirellula baltica* revealed that only 83% of the identified genes were similar to genes of the domain *Bacteria*, while the other 9 and 8% showed the best hits to *Archaea* and *Eukaria*, respectively [6].

Representatives of the *Planctomycetes* are very problematic in cultivation. The first observation of a planctomycete was made during microscopic analysis of pond water in 1924 by Gimesi [7], who described an organism of a fascinating morphology. This organism has been named *Planctomyces bekefii* and is considered the type species of the genus *Planctomyces*, though it remains uncultured up to now. The same uncultured organism was later described by Rasumov as *"Gallionella planctonica"* [8]. The first isolation of these budding, rosette-forming bacteria in a pure culture was achieved in 1973 by Staley, who introduced dilute nutrient media into the practice of oligotrophic bacteria cultivation [9]. Later, a number of media and approaches suitable for the isolation of planctomycetes were developed, which allowed cultures of these bacteria to be obtained from various aquatic habitats [10]. Nonetheless, the number of validly described taxa within the *Planctomycetes* is currently limited to eight species, and six more species of planctomycetes capable of anaerobic ammonium oxidation possess the *"Candidatus"* status, since they have not yet been obtained in a pure culture [11]. Due to the difficulty of planctomycete cultivation, our knowledge of the distribution of these bacteria in natural ecosystems remained limited for a long time. It was thought that these microorganisms are typical of aquatic habitats only. However, with the application of molecular ecology techniques, which allow the direct identification of microorganisms in situ without a need for cultivation, our

[1] Corresponding author; e-mail: dedysh@mail.ru

knowledge of planctomycete ecology has broadened significantly. Planctomycetes were found in various soils [12, 13], anaerobic sediments, and water of marine ecosystems [14], as well as in wastewater treatment bioreactors [15].

In the course of the study of the phylogenetic bacterial diversity in *Sphagnum* peat bogs, we revealed that planctomycetes represent an important component of the microbial community in these ecosystems [16]. This was an unexpected finding, since all described planctomycetes are neutrophilic organisms, while *Sphagnum* peat bogs are characterized by acidic pH (3.5–5.5). The present study was therefore undertaken to check the hypothesis of a wide distribution of planctomycetes in these acidic oligotrophic habitats. The goal of the study was to determine the abundance of members of the *Planctomycetes* in different boreal *Sphagnum* peat bogs and to obtain representative strains of these organisms in a pure culture.

MATERIALS AND METHODS

The peat samples examined in this study were collected from the upper (0–10 cm) oxic layers of three different boreal *Sphagnum* peat bogs in Russia:

1. Oligo-mesotrophic *Sphagnum* peat bog Bakchar, Tomsk oblast, South Vasyugan, Plotnikovo field station of the Institute of Soil Science and Agrochemistry, Siberian Division, Russian Academy of Sciences. The vegetation consists of *Sphagnum angustifolium* and *Sphagnum magellanicum* and patches of *Carex rostrata, Menyantes trifoliata*, and *Equisetum fluviatile.* Peat water had a pH of 4.0 to 4.5 and a conductivity of 40 μS.

2. Ombrotrophic peat bog Obukhovskoe, Yaloslavl oblast, village Obukhovtsevo. The vegetation consists of *Sphagnum angustifolium, Sphagnum fuscum, Carex* spp., *Oxicoccus* sp., and *Vaccinium* sp. Peat water had a pH of 4.2 and a conductivity of 20 μS.

3. Ombrotrophic peat bog of the catchment area of the acidic humic Lake Dubrovskoe, Darwin State Preserve, Vologda oblast. The vegetation consists of *Pinus silvestris, Sphagnum* spp., *Ledum palustre, Andromeda polipholia*, and *Oxicoccus quadripetalus.* Peat water had a pH of 3.75 and a conductivity of 30 μS.

Microbial cells were extracted from peat using a homogenization treatment in BagFilter® disposable sterile plastic bags provided with the Model 100 Mini-Mix laboratory stomacher (Interscience, France). These bags possess an internal filter unit, which divides them into two compartments. This enables separation of the peat suspension from rough plant debris (2–3 mm). Two grams of wet peat were placed into one compartment of such a bag, mixed with 15 ml of sterile water, and treated in the stomacher for 1 min; then, the water enriched with microbial cells was collected from the other bag compartment. The rest of the peat material was mixed with another 15-ml aliquot of water, and the extraction procedure was repeated. Three extraction fractions were obtained from each peat sample, pooled together, and centrifuged at 8500 *g* for 10 min. The pellet was resuspended with sterile water to a final volume of 2 ml, and an 0.5-ml aliquot of this suspension was fixed for 1.5 h with 4% (wt/vol) formaldehyde in phosphate-buffered saline (PBS) (g/l: NaCl, 8.0; KCl, 0.2; Na_2HPO_4, 1.44; NaH_2PO_4, 0.2; pH 7.0). The fixed material was then collected by centrifugation (6600 *g* for 1 min), washed twice with phosphate-buffered saline, resuspended in 0.5 ml of an ethanol–PBS solution (1 : 1, vol/vol), and stored at –20°C until use. Hybridization of the samples with oligonucleotide probes and enumeration of target cells were performed as described before [17]. An equimolar mixture of three oligonucleotide probes, EUB338 + EUB338II + EUB338III (EUB338-mix), was used for the detection of all *Bacteria* members [18], while an equimolar mixture of the probes PLA46 and PLA886 was used for the detection of planctomycete cells [19]. These probes were synthesized and labeled with Cy3 by Syntol (Moscow, Russia).

An earlier developed technique used for cultivation of budding and other oligotrophic bacteria was applied to enrich planctomycetes [10, 20]. Fifteen to twenty sterilized coverslips were inserted vertically for 2/3 of their height into a layer of water agar (15 g agar per l of distilled water) that covered the bottom of a big glass Petri dish. The water agar contained cycloheximide (20 mg per l) to inhibit growth of fungi and protozoa. These plates were then inoculated with peat water (10–15 ml) and incubated in the dark at room temperature for 1–3 months or longer. The biofilms that developed on these coverslips consisted of oligotrophic bacteria capable of adhesion by means of cellular appendages, stalks, and capsules. In the course of incubation, the coverslips were periodically withdrawn, and the microorganisms that had developed on the surface were fixed with a 4% formaldehyde solution (see above). The presence of planctomycete cells in these microbial communities was verified by means of hybridization with the probes PLA46 and PLA886 [19]. When good development of planctomycetes was observed, a few other coverslips were taken out from the agar and washed, and the resulting cell suspension was used for plating onto an agar medium containing (g per liter) KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.05; $CaCl_2 \cdot 2H_2O$, 0.01; Hutner's basal salts [21], 20 ml; *N*-acetylglucosamine, 1.0; Na-ampicillin, 0.2; peptone, 0.1; yeast extract, 0.1; pH 5.8–6.0. When necessary, a glucose solution was added to the medium after sterilization to attain a glucose concentration of 0.5 g/l. In addition, a tenfold diluted agar medium R2A (Difco, France) of pH 5.8–6.0 was used. After 3–4 weeks of incubation, a portion of the cell material from the colonies that appeared on the plates was picked, restreaked on Teflon-coated slides with wells for independent positioning of samples, fixed, hybridized with the probes PLA46 and PLA886, and analyzed microscopically. In case of tar-

Total bacterial cell numbers and cell numbers of planctomycetes detected in *Sphagnum* peat by FISH with the probes EUB338-mix and PLA46 + PLA886, respectively

Peat bog, sampling depth	Number of cells detected by FISH with the probes		Percentage of planctomycete cells in relation to total bacterial number
	EUB338-mix	PLA46 + PLA886	
Bakchar, 0–10 cm	$1.22 \pm 0.34 \times 10^8$	$1.12 \pm 0.34 \times 10^7$	9.2
Obukhovskoe, 0–10 cm	$1.53 \pm 0.18 \times 10^8$	$1.96 \pm 0.49 \times 10^7$	12.8
Peat bog in Darwin State Preserve, 0–5 cm	$1.09 \pm 0.15 \times 10^8$	$4.24 \pm 1.21 \times 10^6$	3.9

get cell detection, the rest of the cell material from the respective colony was restreaked onto fresh media until target organisms were obtained as pure cultures.

DNA was extracted from the isolates using an earlier described modification of the sodium dodecyl sulfate lysis method [22]. PCR-mediated amplification of the 16S rRNA genes was performed using *Bacteria*-specific primers [23] on a PE GeneAmp PCR System 9700 DNA thermal cycler (Perkin-Elmer Applied Biosystems, USA). Nucleotide sequences of the PCR-amplified fragments were determined on an ABI Prism 377A DNA sequencer (Perkin-Elmer Applied Biosystems, USA). Analysis of nucleotide sequences and phylogenetic trees construction were performed by using the ARB software package (http://www.arb-home.de). The statistical significance of the branching points in the trees was calculated using the PHYLIP software package (1000 data resamplings). The 16S rRNA gene sequences determined for strains of planctomycetes from *Sphagnum* peat bogs have been deposited in the GenBank under accession numbers AM162406 and AM162407.

RESULTS

The cell number of planctomycetes in *Sphagnum* peat bogs. Application of the probes PLA46 and PLA886 for the analysis of native peat revealed numerous cells of planctomycetes attached to particles of semidecomposed *Sphagnum* debris. The cells targeted by these probes had spherical or ellipsoid morphology; most of them were assembled in microcolonies. Bright fluorescence of the cells of peat-inhabiting planctomycetes suggested high rRNA content and an active physiological state of these bacteria. Planctomycetes were detected in all *Sphagnum* peat samples taken for this study from peat bogs of different geographic location. As seen from the table, the number of planctomycete cells in the upper (0–10 cm) oxic layers of the peat bog profile was in the range of $0.42–1.96 \times 10^7$ cells per g of wet peat. Thus, planctomycetes comprised 3.9 to 12.8% of the total bacterial number determined by FISH in oxic layers of *Sphagnum* peat bogs.

Enrichment procedure and pure culture isolation. After 3–4 weeks of incubation, the coverslips placed into the water agar, as well as the medium around the coverslips, were covered by microbial biofilms consisting of morphologically diverse organisms. Application of FISH revealed that representatives of the *Planctomycetes* had been actively developing in these biofilms. Brightly shining cell aggregates and microcolonies of planctomycetes were observed in the specimens hybridized with the probes PLA46 and PLA886 (Fig. 1). These bacteria were difficult to identify based on morphological criteria only, since a variety of microorganisms with similar morphology were present in these samples in addition to planctomycetes, which, in their turn, varied in size and cell shape. Restreaking of the cell material from these biofilms onto fresh agar media was performed at the time of most active planctomycete development in one of these enrichment cultures. The colonies that developed on agar media were screened by FISH. The colonies of planctomycetes appeared on media after a long incubation period (from several weeks to several months); they were very small in size (0.5–1 mm) and were detectable with the use of laboratory binocular only. The color of the colonies formed by planctomycetes varied from white and cream to light pink.

Morphological characterization of planctomycetes isolated from peat bogs. As a result of our isolation attempts, three strains of planctomycetes were obtained from *Sphagnum* peat bogs. Strains A10 and MPL7 were isolated from peat bog Bakchar (Tomsk oblast), while strain MOB10 was obtained from peat bog Obukhovskoe (Yaloslavl oblast). Strain A10 had ellipsoid-shaped cells, $2.5–3.2 \times 2.0–2.5$ μm in size, which were assembled in large rosette-like clusters, encompassing up to 40 or more cells (Fig. 2a). On agar media, strain A10 formed raised, light pink, small (0.2–1 mm in diameter) colonies, which were difficult to pick up from the agar surface. The cells of strain MPL7 had ellipsoid-like shape and were 1.8–2.1 μm long and 0.9–1.4 μm wide (Fig. 2b). On agar media, strain MPL7 formed raised colorless colonies 1–2 mm in diameter. The cells of strain MOB10 had spherical shape and were 1.6–2.5 μm in diameter. On agar media, strain MOB10 formed colorless colonies 1 mm in size. The cells of all three strains reproduced by budding,

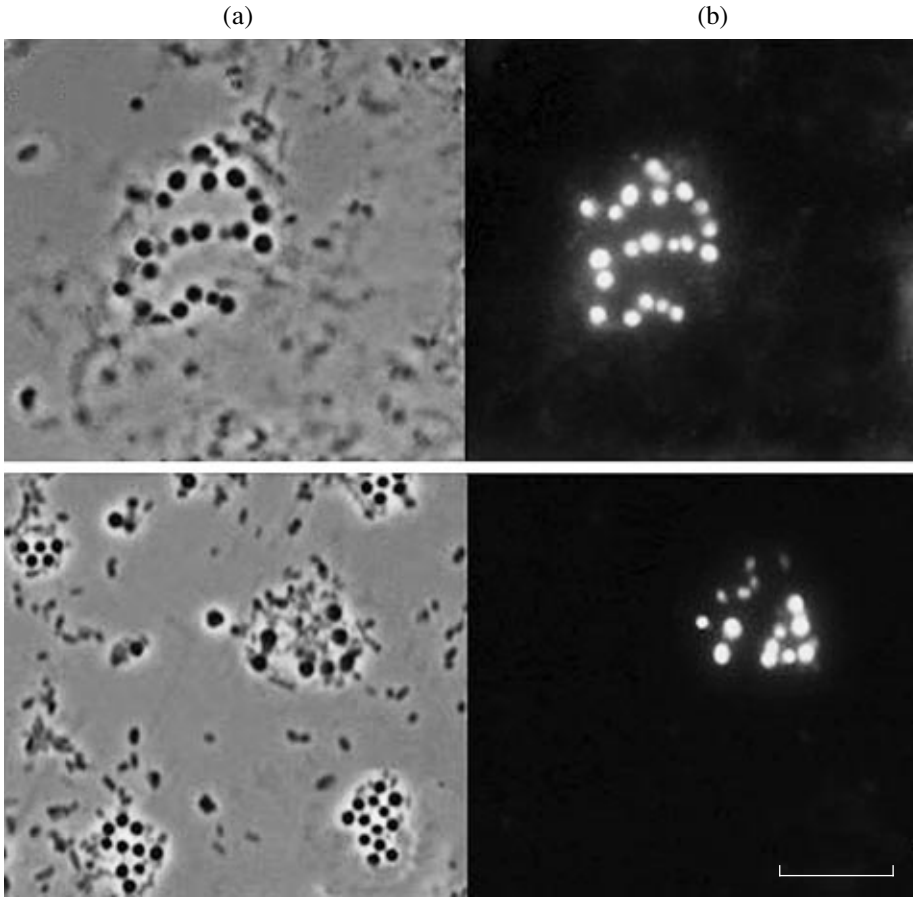

Fig. 1. In situ hybridization of the microbial consortia developing on coverslips with 16S rRNA–targeted oligonucleotide probes specific for representatives of the *Planctomycetes.* a, phase-contrast image; b, epifluorescent micrographs of in situ hybridization with Cy3-labeled probes PLA46 and PLA886. The scale bar, 10 μm.

and daughter cells possessed extremely high motility after their separation from mother cells.

Phylogenetic analysis. The 16S rRNA gene sequences were determined for two isolates obtained from peat bogs, namely, strains A10 and MPL7. Comparison of these sequences with the GenBank database confirmed that these bacteria were members of the *Planctomycetes* (Fig. 3). Strain A10 belongs to a planctomycete lineage defined by the genus *Gemmata* but possesses only 90% sequence similarity to the 16S rRNA gene of *Gemmata obscuriglobus*, the only taxonomically described organism in this group. Strain MPL7 belongs to the phylogenetic group defined by the genus *Planctomyces.* The most closely related organism is *Planctomyces limnophilus*, which however exhibits only 90% sequence similarity to the 16S rRNA gene of strain MPL7. These low values of the 16S rRNA gene sequence similarity between taxonomically described planctomycetes and peat bog isolates suggest that the latter represent novel, yet uncharacterized taxa within the phylum *Planctomycetes.*

DISCUSSION

This study is the first to show that members of the *Planctomycetes* are widely distributed in boreal *Sphagnum* peat bogs and represent one of the numerically significant bacterial groups in upper, oxic layers of the peat bog profile. This is a good example of a microbial community member which might elude attention of microbiologists equipped with traditional cultivation techniques only. In contrast to many other well-studied groups of bacteria, traditional techniques are not suitable for the enumeration of planctomycetes. No medium that would be suitable for the cultivation of all organisms of this phylogenetic group exists, and the growth rates of planctomycetes are very low. As a result, this numerically significant microbial group remains "invisible" to the researcher and can be detected by means of molecular approaches only. It was the FISH method that revealed that planctomycetes might comprise up to 5% of the total microbial cell number in aquatic ecosystems and in activated sludge [19]. The population size of planctomycetes in soils reaches 3×10^9 cells per g of dry soil, comprising up to

7% of the total cell number or 18% of bacterial cells [13]. Taking into account the 95–98% water content in our samples, the abundance of planctomycete cells reached 44×10^8 cells per g of dry peat. Such a large population size suggests the involvement of this group of bacteria in one or several key biogeochemical processes in this ecosystem. The nature of these processes remains to be investigated.

Detection of planctomycetes in acidic *Sphagnum* peat bogs suggests wide physiological diversity within representatives of this phylogenetic group, which, for the most part, consists of uncultured organisms. All currently described planctomycetes are capable of growth in a relatively narrow pH range of 6.0 to 8.5. The only previous case of planctomycete isolation from an acidic peat bog (near Kiel, Germany) was described by Schlesner [10]. However, no properties of this isolate were reported and the strain itself has apparently been lost. According to brief morphological characterization of this organism, it had the same cell morphotype as the isolate MOB10. Thus, the strains obtained in our study represent the first cultures of planctomycetes that were obtained from an acidic habitat and are available for investigation.

Isolation of representatives of the *Planctomycetes* from peat and water of *Sphagnum*-dominated wetlands became possible due to the application of a novel approach that combined traditional, previously developed methods for the isolation of these bacteria [10, 20] with monitoring of all steps of the isolation procedure by means of a molecular technique. The traditional isolation approach is labor-intensive and time-consuming, and its effectiveness is quite low. This is the main reason why this phylogenetic group of bacteria has such a small number of cultured representatives. For a long time, only very few research teams possessed expertise in the isolation of planctomycetes. The use of FISH as a way to visualize the target organisms in complex microbial consortia significantly simplified the isolation procedure and introduced an element of definiteness in the process of screening colonies that appeared on agar media after plating of a sample.

The isolates obtained in this study represent new taxa of planctomycetes. This is supported by their low 16S rRNA gene sequence similarity (90%) to taxonomically described organisms in this group. The morphology of the new isolates is also different from that of phylogenetically related organisms. For example, *Gemmata obscuriglobus* possesses single, spherical cells [24], while cells of strain A10 have an ellipsoid shape and are assembled in large rosettes. The morphology of strain MPL7 also differs significantly from that of *Planctomyces limnophilus*, since the latter possesses cells with highly characteristic stalks [25]. Investigation of the physiological peculiarities and metabolic potential, as well as taxonomic characterization of novel isolates, is of special importance. Compared to well-studied phylogenetic groups of bacteria, such as

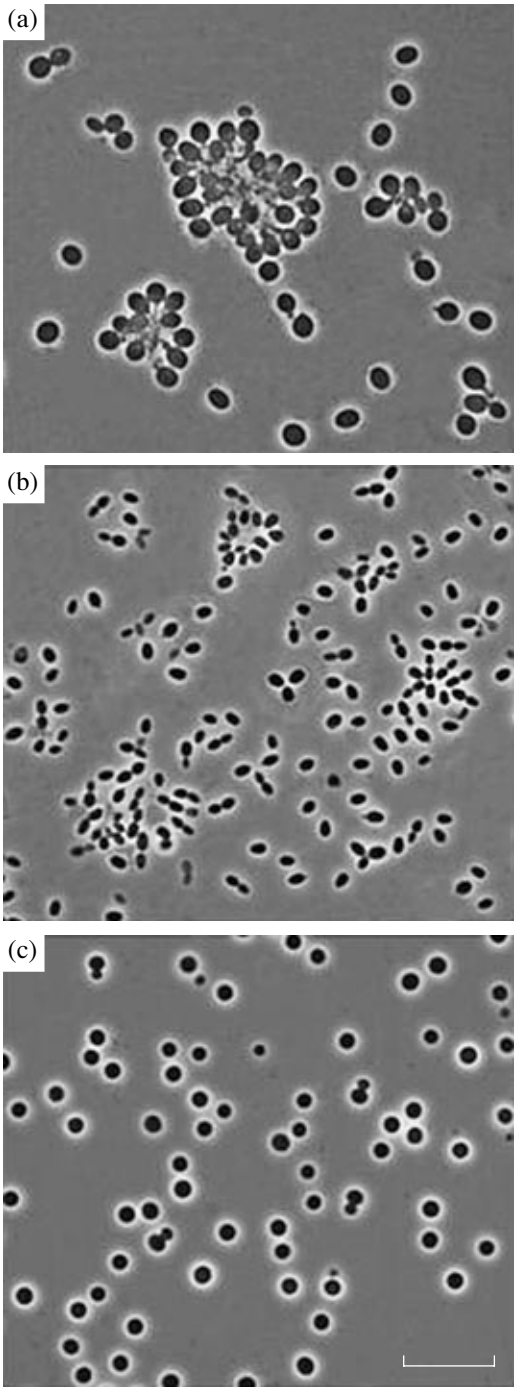

Fig. 2. Cell morphology of strains A10 (a) and MPL7 (b) isolated from *Sphagnum* peat bog Bakchar and of strain MOB10 (c) isolated from peat bog Obukhovskoe. The scale bar, 10 μm.

Proteobacteria, Actinobacteria, or *Firmicutes*, which comprise hundreds and thousands of valid species, the phylum *Planctomycetes* looks like a new book, whose pages describe vast and so far unknown physiological and metabolic diversity. Exploration of this diversity will allow a better understanding of the functional role

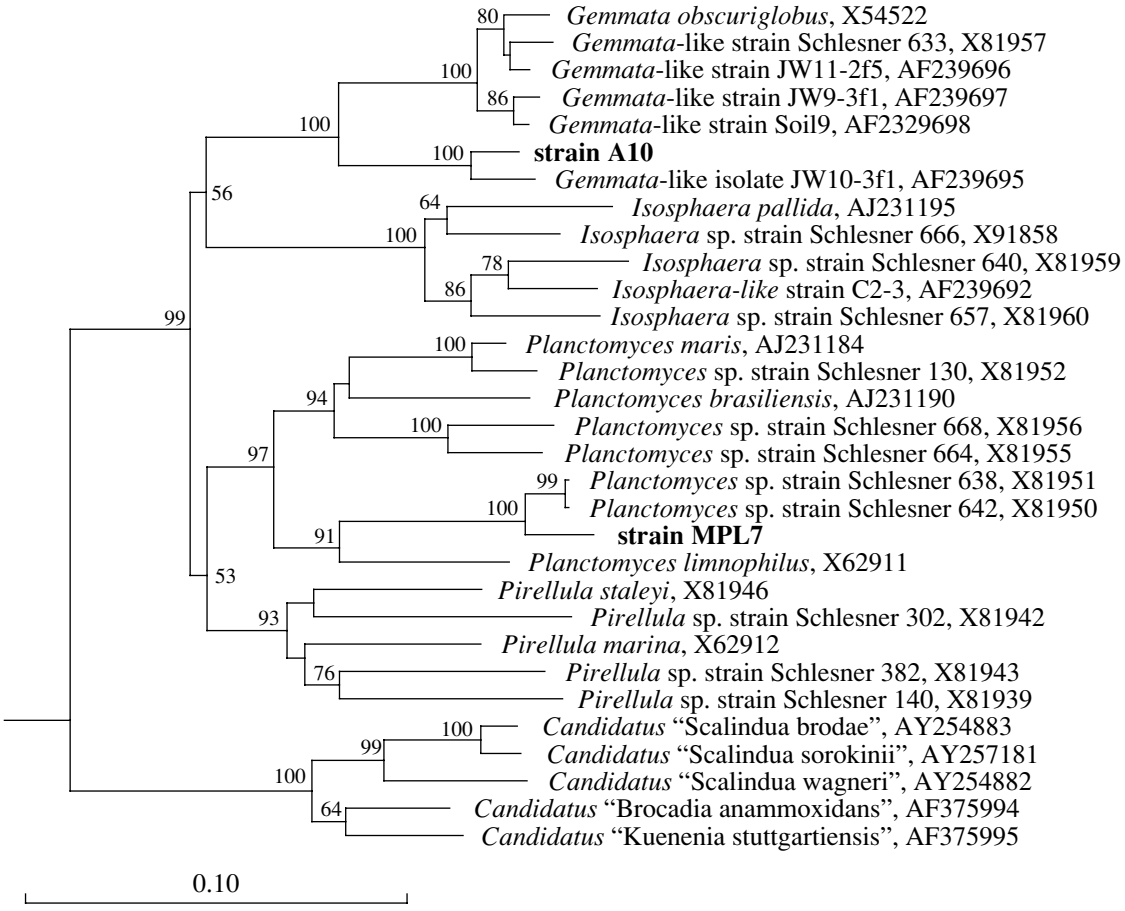

Fig. 3. 16S rRNA–based dendrogram showing phylogenetic relationships of strains A10 and MPL7 to known representatives of the *Planctomycetes.* The 16S rRNA gene sequence of *Chlorobium limicola* (Y10643) was used as an outgroup. The scale bar represents 0.1 substitution per nucleotide position.

of this group of bacteria in the microbial communities of wetland ecosystems.

ACKNOWLEDGMENTS

The authors are greatly indebted to G.A. Zavarzin, who inspired this study and provided many pieces of valuable advice.

This work was supported by the Molecular and Cell Biology program of the Russian Academy of Sciences Presidium, the Program "Leading Scientific Schools," grant no. RI-112/001/027, and by the Russian Foundation for Basic Research, grant no. 04-04-04000.

REFERENCES

1. Fuerst, J.A., The *Planctomycetes*—Emerging Models for Microbial Ecology, Evolution and Cell Biology, *Microbiology* (UK), 1995, vol. 141, pp. 1493–1506.
2. Fuerst, J.A., *Planctomycetes*—a Phylum of Emerging Interest for Microbial Evolution and Ecology, *WFCC Newsletter*, 2004, vol. 38, pp. 1–11.
3. König , H., Schlesner, H., and Hirsch, P., Cell Wall Studies on Budding Bacteria of the *Planctomyces/Pasteuria* Group and on *Prosthecomicrobium* sp., *Arch. Microbiol.*, 1984, vol. 138, pp. 200–205.
4. Lindsay, M.R., Webb, R.I., Strous, M., Jetten, M.S., Butler, M.K., Forde, R.J., and Fuerst, J.A., Cell Compartmentalization in Planctomycetes: Novel Types of Structural Organization for the Bacterial Cell, *Arch. Microbiol.*, 2001, vol. 175, pp. 413–429.
5. Fuerst, J.A., and Webb, R.I., Membrane-Bounded Nucleoid in the Eubacterium *Gemmata obscuriglobus, Proc. Natl. Acad. Sci. USA.*, 1991, vol. 88, pp. 8184–8188.
6. Glöckner, F.O., Kube, M., Bauer, M., Teeling, H., Lombardot, T., Ludwig, W., Gade, D., Beck, A., Borzym, K., Heitmann, K., Rabus, R., Schlesner, H., Amann, R., and Reinhardt, R., Complete Genome Sequence of the Marine Planctomycete *Pirellula* sp. strain 1, *Proc. Natl. Acad. Sci. USA*, 2003, vol. 100, pp. 8298–8303.
7. Gimesi, N., *Planctomyces bekefii* Gim. nov. gen. et sp., *Hydrobiologiai Tanulmányok*, 1924, Budapest: Kiadja a Magyar Ciszterci Rend, pp. 1–8.
8. Krasil'nikov, N.A., *Opredelitel' bakterii i aktinomitsetov* (Manual for Identification of Bacteria and Actino-

mycetes), Moscow–Leningrad: Akad. Nauk SSSR, 1949.

9. Staley, J.T., Budding Bacteria of the *Pasteuria–Blastobacter* Group, *Can. J. Microbiol.*, 1973, vol. 19, pp. 609–614.
10. Schlesner, H., The Development of Media Suitable for the Microorganisms Morphologically Resembling *Planctomyces* spp., *Pirellula* spp., and Other *Planctomycetales* from Various Aquatic Habitats Using Dilute Media, *Syst. Appl. Microbiol.*, 1994, vol. 17, pp. 135–145.
11. Jetten, M.S.M., Cirpus, I., Kartal, B., van Niftrik, L., van de Pas-Schoonen, K.T., Sliekers, O., Haaijer, S., van der Star, W., Schmid, M., van de Vossenberg, J., Schmidt, I., Harhangi, H., van Loosdrecht, M., Kuene, J.G., Op den Camp, H., and Strous, M., 1994–2004: 10 Years of Research on the Anaerobic Oxidation of Ammonium, *Biochem. Soc. Trans.*, 2005, vol. 33, pp. 119–123.
12. Liesack, W. and Stackebrandt, E., Occurrence of Novel Groups of the Domain *Bacteria* as Revealed by Analysis of Genetic Material Isolated from an Australian Terrestrial Environment, *J. Bacteriol.*, 1992, vol. 174, pp. 5072–5078.
13. Zarda, B., Hahn, D., Chatzinotas, A., Schonhuber, W., Neef, A., Amann, R.I., and Zeyer, J., Analysis of Bacterial Community Structure in Bulk Soil by In Situ Hybridization, *Arch. Microbiol.*, 1997, vol. 168, pp. 185–192.
14. Kuypers, M.M.M., Sliekers, A.O., Lavik, G., Schmid, M., Jørgensen, B.B., Kuenen, J.G., Damste, J.S.S., Strous, M., and Jetten, M.S.M., Anaerobic Ammonium Oxidation by Anammox Bacteria in the Black Sea, *Nature*, 2003, vol. 422, pp. 608–611.
15. Strous, M., Fuerst, J.A., Kramer, E.H., Logemann, S., Muyzer, G., van de Pas-Schoonen, K.T., Webb, R., Kuenen, J.C., and Jetten, M.S., Missing Lithotroph Identified as New Planctomycete, *Nature*, 1999, vol. 400, pp. 446–449.
16. Dedysh, S.N., Pankratov, T.A., Belova, S.E., Kulichevskaja, I.S., and Liesack, W., Phylogenetic Analysis and In Situ Identification of Bacterial Community Composition in an Acidic *Sphagnum* Peat Bog, *Appl. Environ. Microbiol.*, 2005, vol. 72, pp. 2110–2117.
17. Pankratov, T.A., Belova, S.E., and Dedysh, S.N., Evaluation of the Phylogenetic Diversity of Prokaryotic Microorganisms in *Sphagnum* Peat Bogs by Means of Fluorescence In Situ Hybridization (FISH), Mikrobiologiya, 2005, vol. 74, no. 6, pp. 831–837 [*Microbiology* [Engl. Transl.), vol. 74, no. 6, pp. 722-728].
18. Daims, H., Brühl, A., Amann, R., Schleifer, K.-H., and Wagner, M., The Domain-Specific Probe EUB338 is Insufficient for the Detection of all *Bacteria*: Development and Evaluation of a More Comprehensive Probe Set, *Syst. Appl. Microbiol.*, 1999, vol. 22, pp. 434–444.
19. Neef, A., Amann, R., Schlesner, H., and Schleifer, K.-H., Monitoring a Widespread Bacterial Group: In Situ Detection of Planctomycetes with 16S rRNA-Targeted Probes, *Microbiology* (UK), 1998, vol. 144, pp. 3257–3266.
20. Hirsch, P., and Müller, M., Methods and Sources for the Enrichment and Isolation of Budding, Nonprosthecate Bacteria from Freshwater, *Microb. Ecol.*, 1986, vol. 12, pp. 331–341.
21. Staley, J.T., Fuerst, J.A., Giovannoni, S., and Schlesner, H., The Order *Planctomycetales* and the Genera *Planctomyces, Pirellula, Gemmata* and *Isosphaera, The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, Balows, A., Trüper, H., Dworkin, M., Harder, W., and Schleifer, K.-H., Eds., New York: Springer-Verlag, 1992, pp. 3710–3731.
22. Dedysh, S.N., Panikov, N.S., and Tiedje, J.M., Acidophilic Methanotrophic Communities from *Sphagnum* Peat Bogs, *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 3, pp. 922–929.
23. Weisburg, W.G., Barns, S.M., Pelletier, D.A., and Lane, D.J., 16S Ribosomal DNA Amplification for Phylogenetic Study, *J. Bacteriol.*, 1991, vol. 173, pp. 697–703.
24. Franzmann, P.D., and Skerman, V.D., *Gemmata obscuriglobus*, a New Genus and Species of the Budding Bacteria, *Antonie van Leeuwenhoek*, 1984, vol. 50, pp. 261–268.
25. Hirsch, P., and Müller, M., *Planctomyces limnophilus* sp. nov., a Stalked and Budding Bacterium from Freshwater, *Syst. Appl. Microbiol.*, 1985, vol. 6, pp. 276–280.